

# Development of Improved Chemically Defined Mammalian Cell Fermentation Processes

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## Abstract

There is an increasing demand for the production of biopharmaceuticals using mammalian cell cultures. Significant effort has been invested in the removal of animal derived raw materials and the subsequent development of chemically defined protein-free processes. To enable economic large-scale production it is desirable to increase process yields and this has led to the requirement for process optimisation. The strategy we have pursued focuses on the development of a robust fed-batch process by manipulation of both the physico-chemical environment and the feeding strategy employed. Following this strategy has led to a 3-fold increase in recombinant antibody production, using a GS-NS0 cell line, in an animal component free, chemically defined process.

## Introduction

- The removal of animal derived raw materials from processes is highly desirable for several reasons.
  - The developing regulatory environment in Europe and the US.
  - Improved process robustness and consistency.
  - Increased purity of harvest material prior to purification.
- Removal of animal derived components can be achieved through the use of microbial peptones or hydrolysates. Whilst somewhat effective, this methodology can result in lot to lot variability. A better approach would be to achieve complete chemical definition of the culture medium.
- Chemically defined processes have been successfully developed for NS0, CHO and hybridoma cell lines. Whilst initial adaptation can result in a small reduction in productivity, this can successfully be recovered through a combination of media and fermenter process optimisation.

## Materials and Methods

- Cell line
  - A GS-NS0 myeloma transfected with GS-linked genes for a chimeric B72.3 IgG antibody against TAG-72 (Bebbington *et al.*, 1992), adapted to protein-free culture in a chemically defined medium.
- Medium and feeds
  - Proprietary chemically defined protein-free medium.
  - Concentrated chemically defined protein-free nutrient feeds.
- Culture conditions
  - 10 L airlift bioreactor, 36.5°C, dissolved oxygen tension 15% of air saturation.
  - Culture pH evaluated as part of the process optimisation.

## Results

- Process optimisation was achieved through development of the nutritional and physico-chemical environment (Table 1, Figures 1 and 2).
- Original process operated at pH 7.3 with a single short duration feed resulted in a product concentration at harvest of 293 mg/L.
- Development of a two continuous feed process coupled with a reduction in culture pH resulted in an increase in culture duration, cumulative cell time and productivity.
- Further rounds of feed development resulted in an improved productivity to in excess of 1 g/L.
- An additional benefit of the optimised process was a reduction in glucose utilisation and lactate accumulation (Figure 3).
  - Simplifies the feeding strategy.
  - Reduction in the requirement for alkali.

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Table 1: Cumulative cell time, productivity, and process duration during optimisation of a model GS-NS0 in chemically defined protein-free medium.

Process	Cumulative cell time (10 <sup>9</sup> cell h/L)	Product concentration (mg/L)	Percent increase in productivity (per stage given in parentheses)	Process duration (d)
Serum-free	640	476	N/A	12
Original	772	293	N/A	12
Optimised v1	1026	589	101	15
Optimised v2	1239	807	175 (37)	16
Optimised v3	1427	1035	253 (28)	16

Figure 1: Productivity of a model GS-NS0 cell line in chemically defined protein-free medium

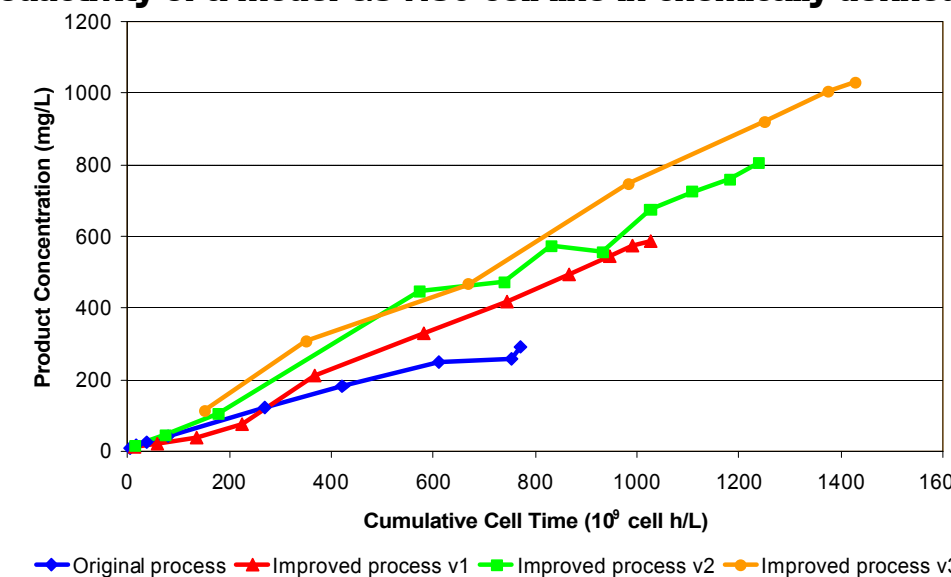


Figure 2: Growth characteristics of a model GS-NS0 cell line in chemically defined protein-free medium

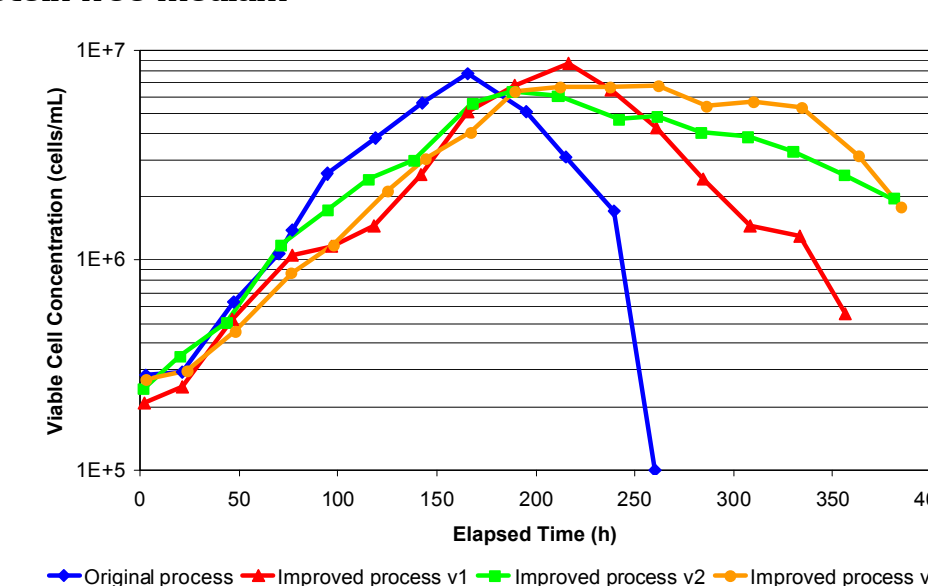
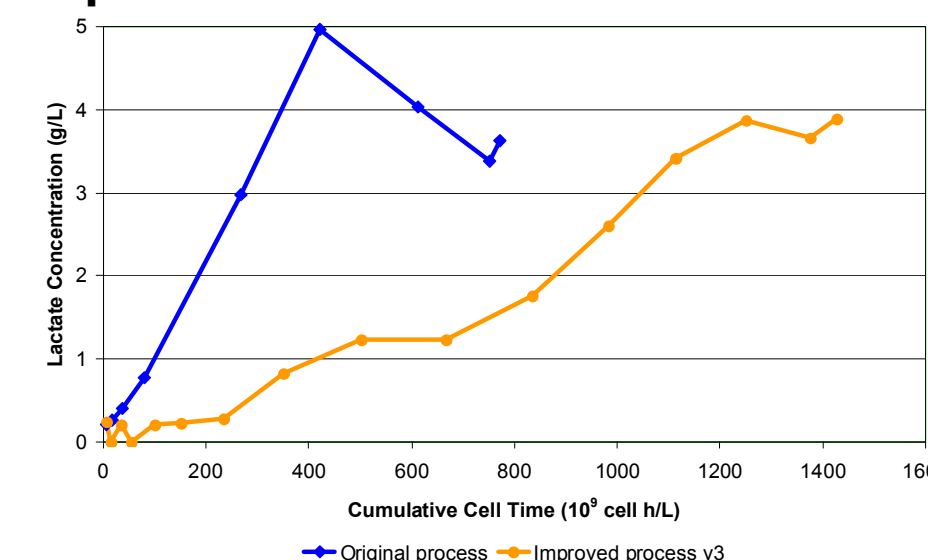


Figure 3: Specific rate of lactate production for a model GS-NS0 cell line in chemically defined protein-free medium



## Downstream processing and throughput benefits

- Increased product concentration and elimination of protein components results in increased purity at harvest, simplifying downstream processing.
  - GS-NS0 protein-containing culture <30% purity.
  - Optimised protein-free culture (v1) 62% - 76% purity.
- Increase in productivity through culture optimisation needs to be assessed in terms of overall production facility throughput.
  - e.g 100% increase in productivity with 25% increase in process duration.
  - increased facility throughput of 70% per annum

## Implementation of optimisation strategy for a GS-CHO cell line

- Similar approach successfully applied to a GS-CHO producing a recombinant antibody.
- Model cell line producing 130 mg/L in serum-free culture adapted to a protein-free, chemically defined medium formulation resulted in an original process producing 334 mg/L.
- Optimisation of the nutritional and physico-chemical environment resulted in an extended decline phase (Figure 4) and an increase in productivity to 585 mg/L (Figure 5).
- Optimised process successfully applied to a new GS-CHO cell line making the same antibody, but created with a different selection protocol, resulting in a productivity of 1917 mg/L.

Figure 4: Growth characteristics of two model GS-CHO cell lines in chemically defined protein-free medium

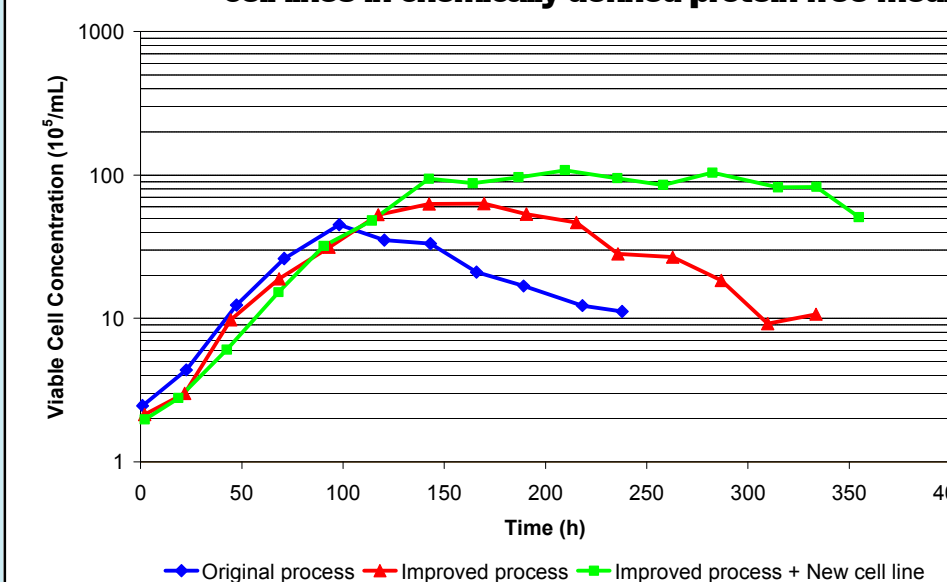
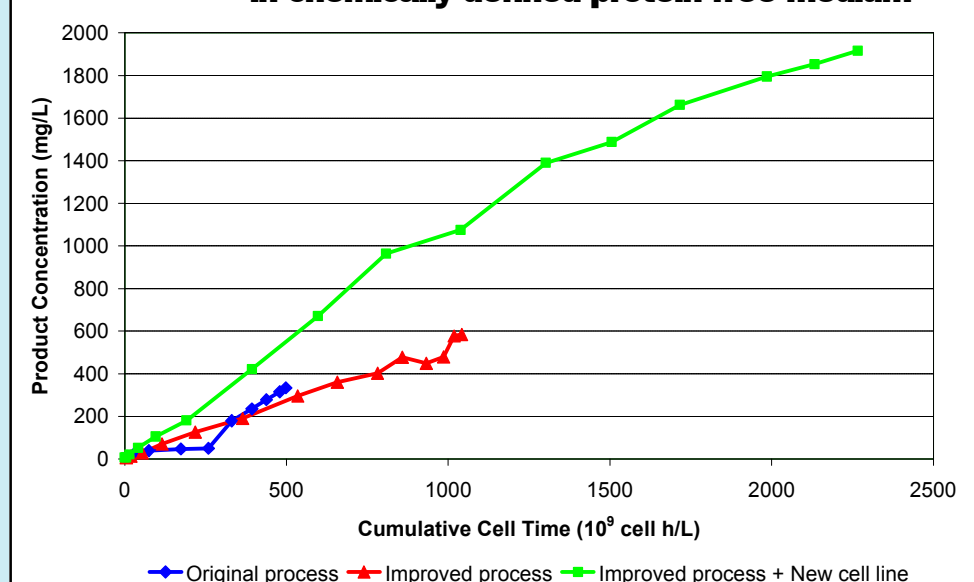


Figure 5: Productivity of two model GS-CHO cell lines in chemically defined protein-free medium



## Conclusions

- GS-NS0 and GS-CHO cell lines can be successfully adapted to growth in chemically defined protein-free media.
- Improvements in fermentation process performance can be achieved through a combination of optimisation of the physico-chemical and nutritional environments and development of improved cell lines.
  - GS-NS0 productivity improved 3-4 fold to >1 g/L.
  - GS-CHO productivity increased 5 fold to 1.9 g/L.
- There are additional benefits to downstream processing.
- Process optimisation must be considered in terms of overall facility throughput.

## References

Bebbington, C.R., Renner, G., Thomson, S., King, D., Abrams, D. & Yarranton, G.T. (1992). *Biotechnology* 10, 169. "High-level expression of a recombinant antibody from myeloma cells using a glutamine synthetase gene as an amplifiable selectable marker".